Reviews of molecular biology of sexually transmitted diseases

Molecular techniques in the diagnosis of human infectious diseases

Ulrich Desselberger, Kathryn Collingham

The availability of clones of nucleic acid (NA) specific for genes of most viral and other microbiological organisms has made possible various approaches to search for such sequences in clinical materials using hybridisation techniques. Radioactive or otherwise labelled NA clones or sequence-specific synthetic oligonucleotides (ONs) act as molecular probes in the search for complementary NA target sequences in the clinical specimens. These procedures have complemented the more traditional diagnostic techniques such as growth in tissue culture (viruses, chlamydia) or in synthetic media (bacteria, fungi, parasites), electron microscopy, examination for antigens by immunofluorescence techniques and serological techniques such as ELISAs, RIAs and other tests looking for specific antibody as the host response to microbial infection.

Several extensive reviews[1-7] and editorials[8] on the topic have appeared. This overview will concentrate on the use of such techniques for the diagnosis of genitourinary tract infections and intrauterine infections and also outline the scope of the technology for the diagnosis of other infectious diseases. As this field is evolving rapidly, it is to be expected that further approaches will become known in the near future.

The principles of the technology

The genetic information of microorganisms is in their nucleic acid (NA), deoxyribonucleic acid (DNA) or ribonucleic acid (RNA; for many viruses). In viral genomes consisting of DNA or RNA the NA can occur in single stranded (ss) or double stranded (ds) forms; the genomes of all higher organised microbes consist of dsDNA (fig 1a).

Inside bacterial cells there are small covalently closed circular pieces of dsDNA called plasmids that

replicate independently of chromosomal DNA. They range in size from 3–5 kilobasepairs (kbp) to 400 kbp (megaplasmids). Such plasmids can be introduced into bacterial cells in vitro by a process called "transformation". Once inside the bacterial cell they replicate using various bacterial enzymes and substrates. Many small plasmids have been used as "cloning vectors". There are numerous ways to convert pieces of genomic NA of all kinds of origin (viruses, bacteria, fungi, microbial parasites, animal or plant cells) into dsDNAs for insertion into cloning vectors and amplification in bacterial cells after transformation.[9]

DNA in its ds state is stable under physiological conditions, but strand separation (denaturation) can be achieved by heating above melting temperature, by raising the pH or by lowering the salt concentration (fig 1b). Conversely, under appropriate conditions, single strands of NA will, provided they are exactly or nearly complementary in sequence, come together ("hybridise") to form a duplex molecule via base pairing (adenine pairs with thymine and cytosine with guanine; in the case of DNA-RNA or RNA-RNA duplexes adenine pairs with uracil) (fig 1c).

Probes are pieces of NA, DNA or RNA, labelled in various ways, which will hybridise to denatured pieces of DNA or RNA present in test samples (clinical specimens of throat swab, faeces, cervical swab, lymphocytes, etc). The preparation of a probe is diagrammatically shown in fig 2. In order to enable binding of the probe to the target both must be ss, that is, denatured. After hybridisation the conditions of washing (temperature, salt concentration, pH) are chosen in a way that only perfect or nearly perfect hybrids will stay together and excess and "mismatched" probe be removed.[10] Conditions of hybridisation can be chosen to result in "high stringency" or "low stringency" binding.[10]

Hybridisation can be carried out with the target NA either in solution or fixed to nitrocellulose or nylon filters which is then called "colony", "dot
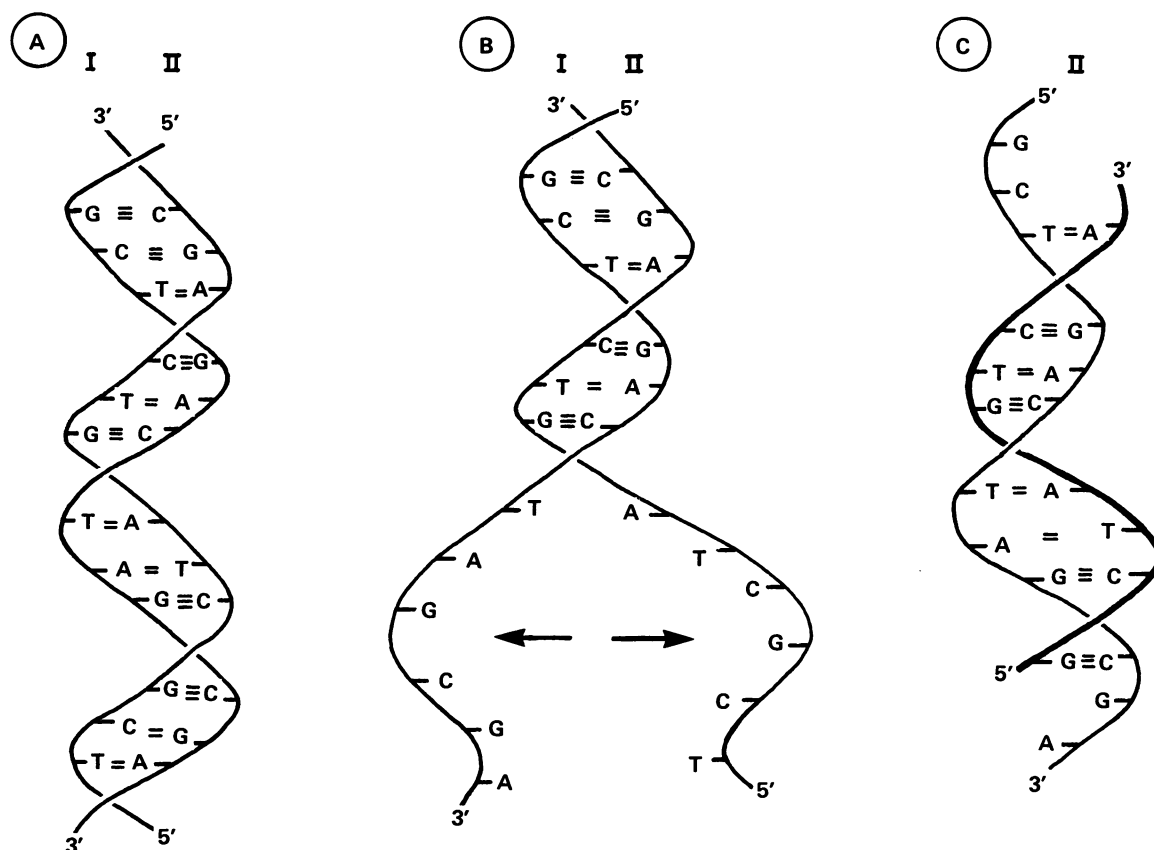
Regional Virus Laboratory, East Birmingham Hospital, Birmingham B9 5ST, UK
Ulrich Desselberger, Kathryn Collingham

Figure 1   Structure of native DNA (a), partially denatured DNA (b) and of target DNA probe DNA hybrids (diagrams). (a) Native ds DNA helix consisting of nucleotide bases adenine (A), guanine (G), thymine (T) and cytosine (C) covalently bonded to deoxyribose molecules which in turn are held together by a phosphodiester backbone connecting the sugar entities in 3' to 5' linkage. (In the diagrams the sugar-phosphate groups are represented by solid lines). The 5' ends of the DNA are phosphorylated, the 3' ends have free 3' hydroxyl groups. Native dsDNAs have 10 nucleotides per turn (so-called "B form"). The 2 strands (I, II) of the DNA helix are in opposite 5' to 3' orientation and are of "complementary" sequence; A is "base-pairing" with T and G with C via hydrogen bonds (G ≡ C pairs have 3 hydrogen bonds against the 2 in A = T pairs and are therefore more stable). In RNA the base T is replaced by uracil (U), which also pairs with A, and the sugar deoxyribose by ribose. (b) Partial separation ("denaturation") of DNA strands due to breakage of hydrogen bonds (by heat, high pH or low ionic strength). (c) Hybridisation of strand II of denatured DNA ("the target") with a complementary piece of DNA ("the probe"; thickened solid line; see Figure 2) under appropriate conditions (heat, salts, etc) to form a new duplex molecule. The hybridised probe is radiolabelled continuously or at the ends (detection by scintillation counting or autoradiography), covalently linked with enzymes (phsphatase or peroxidase, detected by reaction with substrates) or with biotin (detected by enzyme-labelled streptavidin). Both the target and the probe can be DNA or RNA (DNA-RNA and RNA-RNA hybrids not shown).

blot" or "slot blot hybridisation". Pieces of nucleic acid can also be separated electrophoretically on gels and subsequently blotted or electrophoresed onto membranes which are used for hybridisation ("Southern blots" for DNA, "Northern blots" for RNA).[9] Hybrids which have formed in solution are adsorbed to hydroxyapatite and separated by centrifugation from the hybridisation mixture. Hybridisation can also be carried out in situ in order to localise certain sequences in cells or subcellular compartments.[11]

Probes can be of various structure and be labelled in various ways. They are either dsDNA (cloned DNA), ssDNA (obtained from M13 clones[12] or fully synthesised oligonucleotides) or ssRNA (obtained as transcripts from dsDNA vectors possessing bacteriophage transcriptional promoters for SP6 or T7 polymerases[13] (fig 3) or from a synthetic partially ds (14) or ss (14a) oligonucleotide possessing a polymerase promoter). Probes are continuously labelled either by nick-translation (dsDNA) incorporating ^{32}P-dNTPs[15] or biotinylated dNTPs[16] using DNA polymerase I[15] (fig 4), by T4 DNA polymerase[17] (fig 5), or by the Klenow fragment of polymerase I (fig 6).
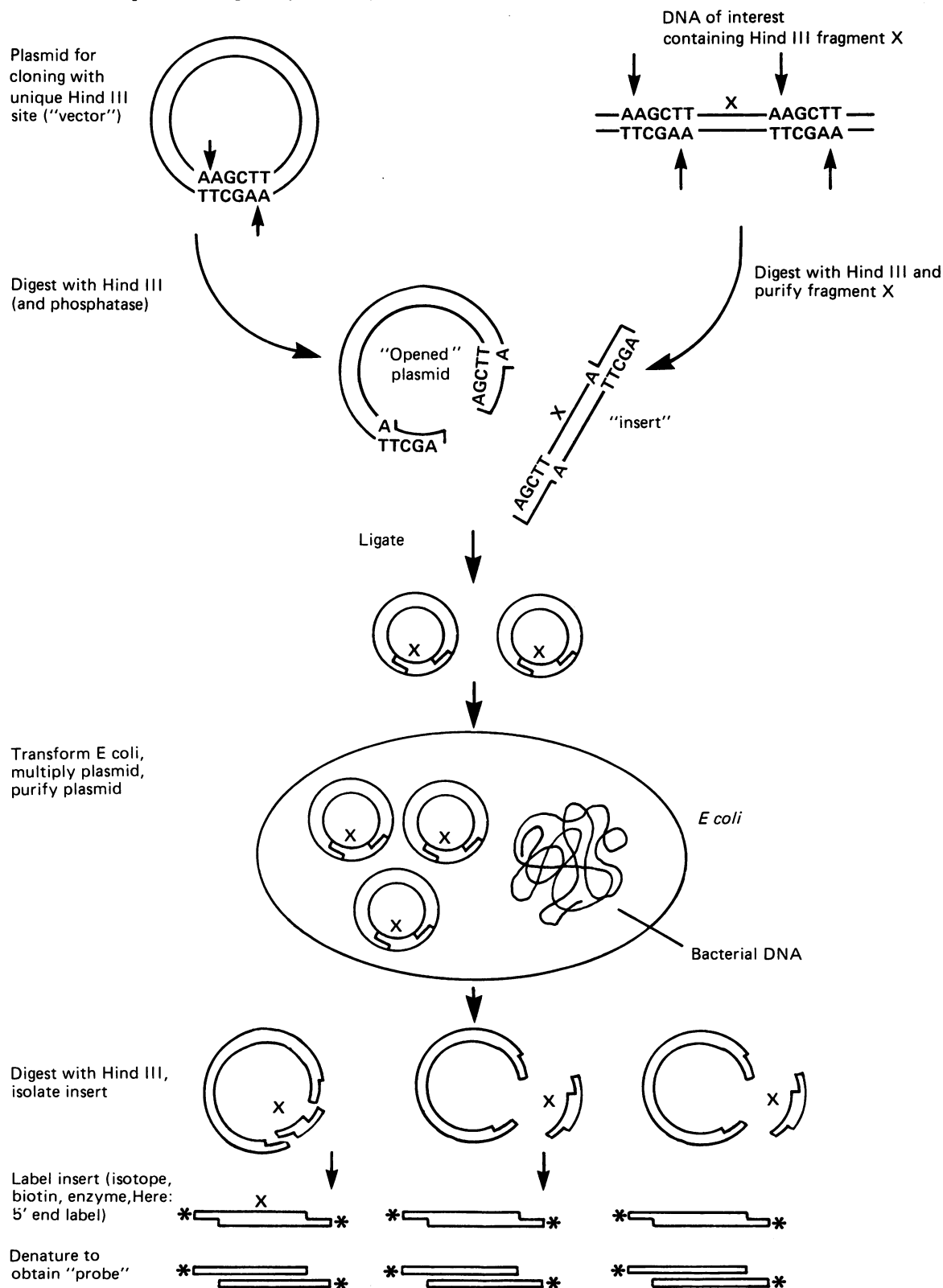
Plasmid for cloning with unique Hind III site ("vector")

DNA of interest containing Hind III fragment X

—AAGCTT —X— AAGCTT —
—TTCGAA ———— TTCGAA —

AAGCTT
TTCGAA

Digest with Hind III (and phosphatase)

Digest with Hind III and purify fragment X

"Opened" plasmid

AGCTT
A

A
TTCGA

X

AGCTT
A

A
TTCGA

"insert"

Ligate

X   X

Transform E coli, multiply plasmid, purify plasmid

X   X

X

E coli

Bacterial DNA

Digest with Hind III, isolate insert

X   X   X

Label insert (isotope, biotin, enzyme, Here: 5' end label)

X
━━━━   *━━━━*   *━━━━*

Denature to obtain "probe"

━━━   *━━━*   *━━━*
━━━   *━━━*   *━━━*

Figure 2 Preparation of a DNA probe. A restriction endonuclease fragment of interest (here: Hind III fragment X) is ligated ("inserted") into the unique Hind III site of a plasmid ("cloning vector"). The vector containing the insert is then transformed into E coli and amplified, leading to bacterial lysis. After purification of the plasmid its insert is liberated by Hind III digestion, isolated, labelled (here: 5' end label, for example with ^{32}P) and denatured. It is now ready as "probe" for reaction with a complementary "target" DNA in a hybridisation reaction (figure 1c).
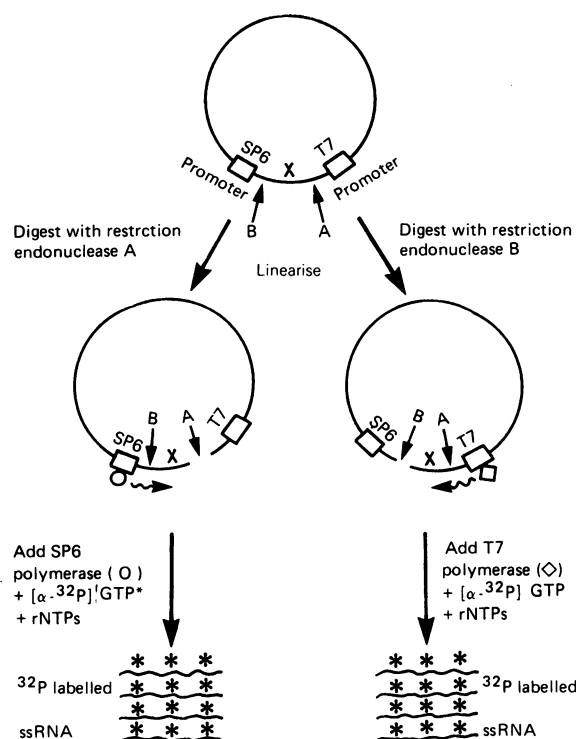
Figure 3   The SP6/T7 polymerase system. The vector is a derivative of plasmid pBR322 into which the promoter sequences of the SP6 and T7 polymerases have been engineered. DNA sequences of interest (here: X) can be inserted between the two promoters into a multiple cloning site (not shown) using standard techniques (Ref 9 and Figure 2). Linearisation of this construct with restriction endonucleases A or B and addition of SP6 of T7 polymerases, respectively, in the presence of α-^{32}P-labelled and unlabelled ribo-NTPs will result in the production of multiple ssRNA transcripts of high specific radioactivity as probes. The system is very efficient, yielding up to 10 µg product from 1 µg template. Template DNA is removed by treatment with RNase-free DNase I, and the transcripts purified by Sephadex chromatography. The SP6 and T7 ssRNA products are of opposite "sense" and can be used to differentiate between genomic DNA and mRNA templates (eg by in situ hybridisation).

Alternatively 5′ end labelling with gamma-^{32}P-ATP and T4 polynucleotide kinase is used for short DNA fragments or synthetic oligonucleotides.[18]

The extent of hybridisation is monitored by autoradiography of filters or of tissue sections treated in situ. Dots from filters can also be cut out and counted in a liquid scintillation counter.

For various reasons (safety, costs, stability), non-radioactive methods of labelling probes have been developed.[16 19 20] The most frequently used is the incorporation of biotinylated nucleotides (such as biotin-11-dUTP, figure 7) into probes.[16 19 20] Hybridised biotinylated NA is detected by an avidin-
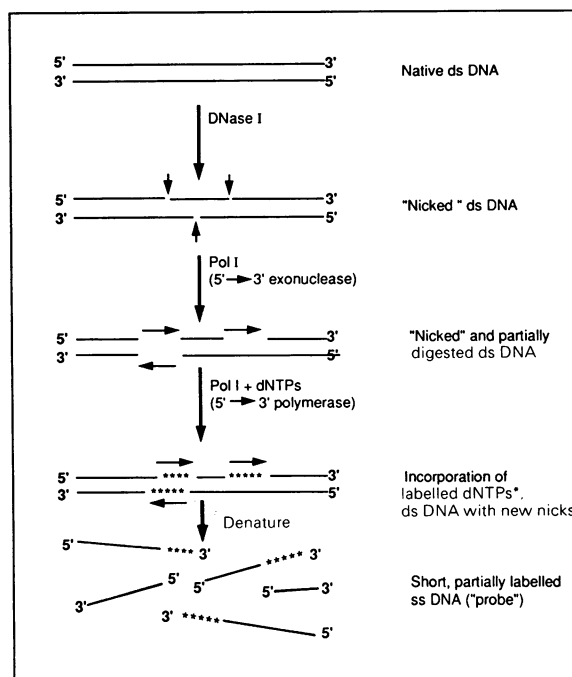

Figure 4   Labelling of probes by "nick-translation" of dsDNA with DNase I and E coli DNA polymerase I. dNTPs can carry a radioactive label (^{32}P, ^{14}C, ^{3}H) or can be biotinylated.
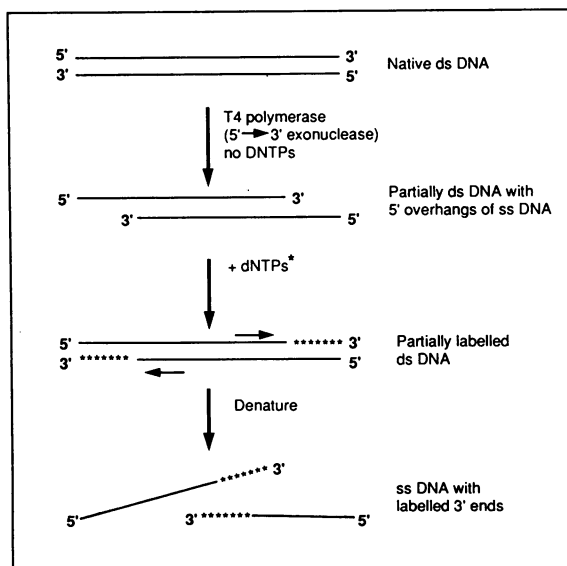

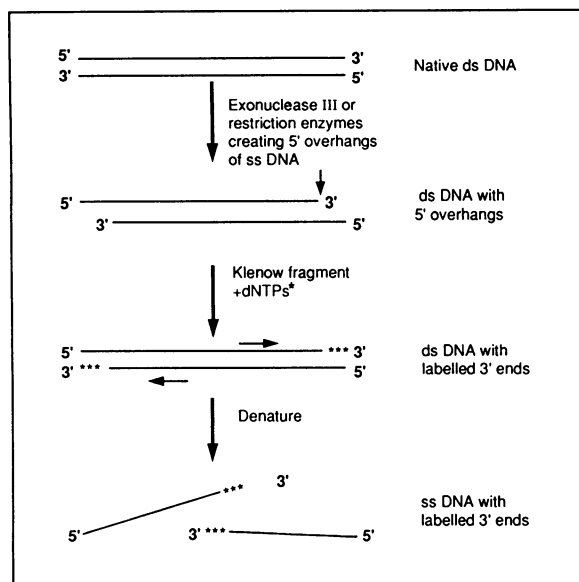*Figure 5   Labelling of DNA probes using T4 DNA polymerase.*

Figure 6   Labelling of DNA probes using the large fragment (Klenow) of E coli DNA polymerase I.

enzyme complex, wherein avidin binds with high affinity to biotin. The enzymes most frequently used in the complex are alkaline phosphatase, horseradish peroxidase and beta-D-galastosidase which convert colourless substrates into coloured products; these are detected either by eye or quantitated photometrically.[19] Biotinylation of oligonucleotides has also been accomplished at both the 3′ and 5′ ends.[21] NA probes have been directly end-labelled with enzymes.[22][23]This does not seem to affect the hybridisation kinetics.

An elegant NA sandwich hybridisation was introduced by Virtanen *et al.*[24] Here the target NA is immobilised to a filter by a capture NA and then probed by a labelled NA complementary to a part of the target. which does not interact with the capture NA.
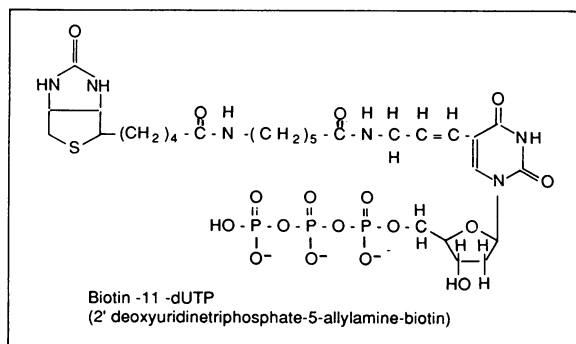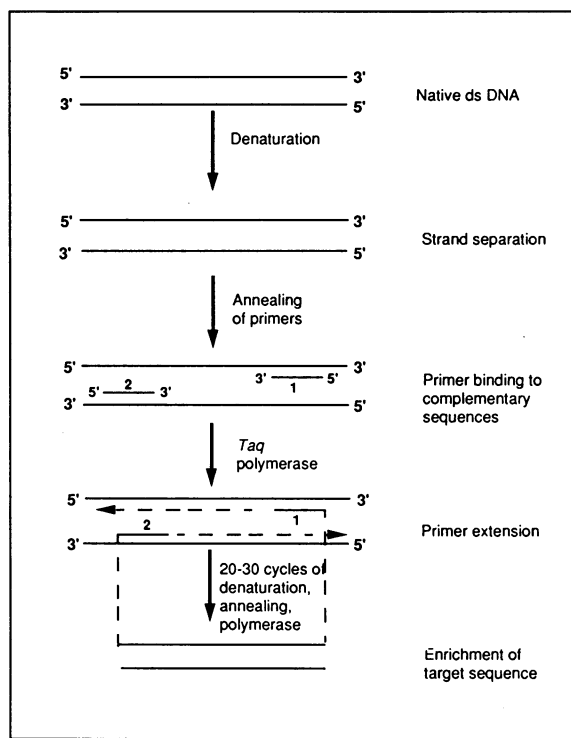

Figure 8   The polymerase chain reaction (PCR). The starting material for PCR, the "target sequence" of dsDNA is denatured by heating (typically 95°C, initially 60 seconds, subsequently 15 seconds), then primers complementary to opposite strands in the desired distance of target DNA are annealed (55°C, 30 seconds) and then extended with Thermus aquaticus (Taq) polymerase (72°C, 90–240 seconds). This cycle is repeated 20–30 times leading to amplification of the target sequence by up to 10⁶-fold. Many modifications of the original PCR have been described. RNA can also be amplified by PCR after an initial reverse transcriptase step.

Various modifications and extensions of these procedures for hybridisation reactions have been reviewed by Jablonski.[25]

Advantages and disadvantages of DNA probes
Before discussing some applications of the technology to specific pathogens, the relative advantages and disadvantages of hybridisation techniques compared to more classical techniques should be considered.

A major advantage of the hybridisation technology is in the detection of genomes of microorganisms which are present in a latent state (members of the herpesvirus family) or for which cultivation in vitro has proven to be difficult (for example enteroadenoviruses) or, as yet not possible (human papillomaviruses (HPV), human parvovirus B19 (HParV), hepatitis B viruses (HBV) and other hepatotropic viruses). For several organisms the use of a probe

Biotin -11 -dUTP
(2′ deoxyuridinetriphosphate-5-allylamine-biotin)

Figure 7   Structure of biotin-11-dUTP.

provides an earlier diagnosis than growth in tissue culture (such as human cytomegalovirus, HCMV) or cultivation on cell-free substrates (mycobacteria). The technique can also be used in situ in formalin-fixed tissues and exfoliated cells.

Disadvantages can be that only a limited number of microorganisms is detected in a specimen, depending on the composition of the probe; that detection of the genome of a microorganism is open to interpretation as to its activity (latent or productive infection); that there is the possibility of cross-reactivity of the probe with cellular (chromosomal and ribosomal) sequences present in the specimen; that the quantity of microorganisms in the specimen may be low and below the limit of detection by a probe (giving rise to false negative results); that there are no established external quality controls for the procedures yet; that the initial costs are high; and that ^{32}P- or ^{125}I-labelled probes have a relatively short shelf life, and their use requires special safety precautions.

However, most of the disadvantages mentioned may become less significant in the near future. There is work ongoing to incorporate several probes into one hybridisation assay and to develop probes which are highly cross-reactive within a group of microorganisms.[26] Probing for mRNA as opposed to genomic NA and for such mRNAs preferentially made in productive infections allows some statements to be made about the degree of replicative activity of the microorganism.[27] The problem of false-positivity of the probe ("spurious" cross-reactivity wth cellular NA sequences) is well recognised and can be minimised.[28] There are several and some very powerful procedures to amplify genomic NA before hybridisation. The polymerase chain reaction (PCR)[29-31] (diagrammatically shown in fig 8) amplifies specific DNA in vitro based on repeated cycles of denaturation by heat (>90°C), annealing of ON primers (at 50°C) and primer extension at 60–70°C using the heat-stable DNA polymerase of *Thermus aquaticus* (*Taq* polymerase). Twenty to thirty such cycles can result in 100,000-fold to 1 million-fold amplification of the target sequence. The procedure is so sensitive (detection of 1 target sequence in the DNA of 10^6 cells) that contamination can be a major problem.

The problems of quality control and relative costs of DNA probes need to be more precisely defined.

APPLICATIONS OF DNA PROBES TO DIAGNOSIS OF INFECTIOUS DISEASES
Herpes simplex virus (HSV)
Infection with HSV type 1 and type 2 (large DNA viruses) are very common in man. After initial infection a latent virus-host cell relationship is established, mainly in sensory ganglion cells from which frequent reactivations can occur. HSV causes keratitis, encephalitis, recurrent cold sores and recurrent genital herpes. In the immunocom-

promised host, HSV infection often causes severe disease. (For details of the virology and epidemiology of HSV and the viruses discussed below see ref 32).

Although HSV DNA-specific hybridisation techniques are unlikely to supersede culturing and immunofluorescence techniques, they have been applied to viral diagnosis. The choice of appropriate probes has allowed differentiation between HSV1 and HSV2 infections,[33-35] recognition of genital HSV transmission[36] and recognition of mRNA transcripts during latency.[37 38]

It should be mentioned that large HSV-"specific" DNA probes shown cross-reactivity with mammalian cellular sequences;[39 40] therefore appropriate controls are essential and shorter probes preferable.

Papillomaviruses (HPV)
Papillomaviruses are small DNA viruses replicating in various mammalian species (cattle, rabbits) and in man. At present, there have been 60 types of human papillomaviruses (HPVs) described which are differentiated on the basis of less than 50% cross-hybridisation of their NAs under stringent conditions.[32 41] As the HPVs cannot be grown in tissue culture, hybridisation techniques have been essential for their classification. HPVs have been found to be associated with a number of cutaneous and mucosal benign lesions (common warts, plantar warts, epidermodysplasia verruciformis, condylomata acuminata) but special interest has arisen from finding an association of certain HPV types (mainly types 16 and 18, but also 6 and 11 and many others[41]) with precancerous lesions (cervical intraepithelial neoplasia (CIN)) and with cancer of the cervix and vulva.[42-44]

On the other hand, HPV DNA has been found in normal tissue adjacent to anogenital condylomata and only in half of CIN and carcinomatous lesions.[45 46] Sixty percent of cervical lesions with abnormal cytology contain HPV types other than 6, 11, 16 or 18.[47] Recent findings indicate that HPV DNA is present frequently in completely healthy carriers.[48-52] Clear epidemiological evidence for the aetiological role of human papillomaviruses in cervical neoplasia is still lacking.[53 54] It is speculated that the amount of HPV DNA can decrease dramatically as lesions develop and that HPV is one of several factors involved in tumour development.[55 56]

Human Cytomegalovirus (HCMV)
HCMV, a genus of the herpesvirus family, is a frequent viral cause of intrauterine infection resulting in congenital malformation or later sequelae.[57] In the immunocompromised patient HCMV can cause a wide range of clinical symptoms (pneumonia, retinitis, hepatitis, encephalitis, colitis). Diagnosis by growth in tissue culture may take several weeks,

although testing for an immediate early antigen using monoclonal antibody allows a diagnosis after 24 hours in many cases.[58] Great care has to be taken in the selection of the probe as wide parts of the CMV genome cross-react with human DNA.[59] With these precautions, latent or reactivated HCMV can be detected in lymphocytes and other tissues.[27 60] Recently, the polymerase chain reaction (PCR) has been used to amplify HCMV-specific sequences; a high degree of sensitivity and concordance with viral isolation data from different sources (infected fibroblasts, peripheral leucocytes and urines) was achieved.[61]

Human Immunodeficiency Virus (HIV)

By the end of 1989 approximately 200,000 cases of acquired immunodeficiency syndrome (AIDS) have been recorded worldwide (of whom more than half have died), and worldwide several million people are thought to be infected with HIV, the causative agent of AIDS. The virus, a member of the retroviridae family, is transmitted by blood and sexual contact, and homosexuals, intravenous drug abusers and haemophiliacs (before universal screening of blood donations in 1985) have been the major risk groups so far. The rate of heterosexual transmission is increasing at an alarming rate. The infection is diagnosed by testing for HIV-specific antibody (particle agglutination tests, ELISAs, Western blots) or by culturing the virus. Although culture is carried out in many laboratories, the method is cumbersome and needs special safety precautions.[62–64] Hybridisation techniques have been used to detect HIV genomes in lymphocytes[65–66] and nerve tissue.[67] Recently the PCR has been applied in order to detect HIV-specific sequences in lymphocytes and tissues and has done so with a high degree of sensitivity.[63 68 69] Such a test meets a special need as the HIV-specific seroconversion occurs several months or more after the initial infection[70] and the virus has in some cases been transmitted by blood which was screened negative for HIV-specific antibody.[71] Several groups have detected HIV infection by PCR in seronegative individuals,[72–74] mainly in the high risk seronegative sexual partners of HIV-infected people and in infants born to seropositive mothers.[73 76] From such data it was estimated that 95% of HIV-infected individuals do seroconvert within 5–8 months.[77] Once PCR is sufficiently standardised and properly controlled, it may become more widely applied as a direct test for HIV infection. Besides these applied aspects it is clear that hybridisation and PCR techniques have greatly augmented our knowledge of HIV pathogenesis and variation.[78 79]

Hepatitis viruses

Infectious hepatitis can be distinguished into at least five forms: hepatitis A to E. Hepatitis A and B viruses (HAV, HBV) are well characterised and a number of serological tests for specific antibodies (HBsAb, HBeAb, HBcAb; HAVAb) and antigens (HBsAg, HBeAg; HAVAg) are available. HBV, a small DNA virus, is very fastidious, and there is no easy way to grow it in tissue culture. In approximately 5% of human infections a chronic infection ("carrier state") results, and there are an estimated 200 million HBV carriers worldwide who act as a reservoir for transmission. HBV carriers have a 200-fold increased risk of developing hepatocellular carcinoma. HAV is a small RNA virus. Infection is mostly by the faecaloral route, no chronic carrier state or disease results. Infectious forms of hepatitis not caused by HAV or HBV were called nonA,nonB hepatitis until very recently when a serological test for antibody against a parenterally transmitted virus called hepatitis C virus (HCV) became available;[80] but the significance of positive results from this test remains to be defined.

HBV infections are mainly transmitted sexually and by infected blood. A further major route of transmission is from carrier mother to child during birth. The inability to grow HBV led investigators to search for the presence of the HBV genome in serum and tissues. HBV possesses a genome of partially dsDNA which has been cloned and sequenced for several subtypes (for example ref 81). Molecular probes derived from these sequences were used to show the presence of HBV DNA in serum,[82] hepatocytes[83] and various other organs and peripheral leucocytes.[84–91] Cloned DNA probes were successfully replaced by ON probes.[92 93]

Hybridisation techniques have shown the presence of HBV DNA in serum of HBsAg- and HBeAg-positive individuals and also in sera of some patients testing negative for these markers. The technology has also revealed the presence of HBV in non-hepatocytes including leucocytes. These findings provide very important criteria for infectivity of the individual. Furthermore, measuring HBV DNA is an important tool in evaluating treatment of chronically HBV-infected individuals, for example by interferon.[94]

The delta agent (also named HDV) consists of closed circles of naked RNA which is intensely internally base-paired. Infection with HDV is only found in association (co- or superinfection) with HBV infection; HDV parasitises capsid and envelope of HBV. The only HDV-specific expressed antigen is delta antigen which is incorporated into the capsid. Delta antigen and specific antibody can be measured. In addition, an HDV-specific RNA:RNA hybridisation procedure has been published[95] which will shed more light on mechanisms of chronicity and latency.

Hepatitis C virus genome has recently been detected by PCR in the sera of patients with chronic nonA–nonB hepatitis.[96]

Human parvovirus B19 (HParV B19)

HParV B19 is recognised as the causative agent of erythema infectiosum or fifth disease.[97] Most people are infected during childhood, but a significant number (20–40%) are still susceptible as adults. In adulthood the infection may be asymptomatic or present with fever, headache, myalgia and often arthritis/arthralgia,[98] particularly in adult women. Individuals with sickle cell anaemia or other inherited disorders of erythrocytes develop a severe aplastic crisis after infection with HParV B19.[99 100] HParV B19 is not sexually transmitted, but infections during pregnancy have been associated with hydrops fetalis, stillbirth and spontaneous abortion.[101-105]

The virus, a small ssDNA virus, does not easily grow in tissue culture. Therefore, normally the diagnosis of acute infection is made by testing for IgM antibody specific for HParV B19 in a RIA. However, there is a need to test for the presence of this virus in cases of spontaneous abortions, and dot hybridisation tests using labelled fragments of cloned HParV B19 DNA or ssRNA transcripts as probes have been developed.[106-109] Recently, application of the PCR technology has increased the sensitivity of testing for the presence of parvovirus DNA in clinical specimens enormously.[110 101]

Chlamydia

Chlamydia trachomatis is among the most frequent causes of sexually transmitted diseases.[112-114] There are 15 serotypes causing primary urethritis and cervicitis with the complications of epididymitis, proctitis, endometritis and salpingitis. In newborns they can cause severe conjunctivitis and pneumonia.

Chlamydiae are obligate intracellular parasites. Laboratory diagnosis is mainly by cell culture, but also by IFT and ELISA. Several groups have described hybridisation tests to identify chlamydial DNA.[115-121] The probes are complementary to chromosomal DNA,[115] cryptic plasmids[118-120] or ribosomal RNA.[121] All probes were cross-reactive throughout serotypes but otherwise specific.[122] The DNA hybridisation assay is slightly less sensitive but as specific as cell culture.[120-122] False negative results with DNA hybridisation were from specimens with low infectivity (less than 100 inclusion-forming units; ref 122).

Gonococci

In cases of male urethritis gonococci have been detected by hybridisation using a probe specific for a gonococcal plasmid.[123] Comparison of such results with those of culture of the microorganism must be carried out with caution as culture itself may not detect 100% of the infections.[124]

Diagnostic nucleic acid probes for other infectious agents

Molecular techniques have been used for the detection in clinical specimens of various other microorganisms: Viruses (adenoviruses,[24 125-128] varicella-zoster virus,[129 130] Epstein-Barr virus,[131] rotaviruses[132]), bacteria (enteropathogenic E coli, shigella, salmonella, campylobacter,[133] vibrio,[134] legionella, mycobacterium, mycoplasma[135]). The probes against the pathogenic gut bacteria are mainly based on reaction with toxin or virulence plasmid genes, whereas probes against the respiratory pathogens are based on sequences found in ribosomal RNA of these microorganisms. The probes are highly specific and in many cases highly convergent with the results from cultures which normally take much more time. Infections with parasitic agents (plasmodium, babesia,[136] leishmania[137]) can also be diagnosed by DNA probes which are preferentially based on so-called repetitive sequences in the chromosomes or mitochondria of these parasites.

Summary and outlook

Nucleic acid probes of different structure and labelled in various ways are increasingly used for the diagnosis of viral, bacterial and parasitic infections. The technology complements rather than replaces more classical diagnostic techniques (culture and serology) and has provided new insight into the pathogenesis and epidemiology of infectious diseases. Its usefulness for other purposes, such as susceptibility and resistance testing of microorganisms[138] is being explored.

1 Bornkamm GW, Desgranges C, Gissmann L. Nucleic acid hybridization for the detection of viral genomes. *Current Topics Microbiol Immunol* 1983;104:287–98.
2 Kulski JK, Norval M. Nucleic acid probes in diagnosis of viral diseases in man. *Arch Virol* 1985;83:3–15.
3 Schachter J. Rapid diagnosis of sexually transmitted diseases. *Diagn Microbiol Infect Dis* 1986;4:185–9.
4 Horn JE, Quinn T, Hammer M, Palmer L, Falkow S. Use of nucleic acid probes for the detection of sexually transmitted infectious agents. *Diagn Microbiol Infect Dis* 1986;4:5101–9.
5 Norval M, Bingham RW. Advances in the use of nucleic acid probes in diagnosis of viral diseases of man. *Arch Virol* 1987;97:151–65.
6 Tenover FC. Diagnostic deoxyribonucleic acid probes for infectious disease. *Clin Microbiol Rev* 1988;1:82–101.
7 Tenover FC (ed). *DNA Probes for Infectious Diseases*. Boca Raton, Florida, CRC Press, 1989.
8 Editorial. DNA probes—where are they taking us? *Eur J Clin Microbiol Inf Dis* 1988;7:457–9.
Editorial. DNA technology and rapid diagnosis of infection. *Lancet* 1989;ii:897–8.
9 Sambrook J, Fritsch EF, Maniatis T. *Molecular Cloning: A Laboratory Handbook*, 2nd ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor NY, 1989.
10 Hames BD, Higgins SJ, (eds). *Nucleic Acid Hybridization, A Practical Approach*. Oxford, IRL Press, 1985.
11 Brigati DJ, Myerson D, Leary JL, et al. Detection of viral genomes in cultured cells and paraffin-embedded tissue sections using biotin-labelled hybridization probes. *Virology* 1983;126:32–50.
12 Norrander J, Kempe T, Messing J. A new pair of M13 vectors for selecting either strand of a double digest restriction fragment. *Gene* 1983;26:101–6.
13 Melton DA, Krieg PA, Rebagliati MR, Maniatis T, Zinn K,

Green MR. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res* 1984;12:7035–56.

14 Milligan YF, Groebe DR, Witherell GW, Uhlenbeck OL. Oligonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates. *Nucleic Acids Res* 1987; 15:8783–98.

14a Sharmeen L, Taylor J. Enzymatic synthesis of RNA oligonucleotides. *Nucleic Acids Res* 1987;15:6705–11.

15 Rigby PWJ, Dieckmann M, Rhodes C, Berg P. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick-translation with DNA polymerase I. *J Mol Biol* 1977;113:237–51.

16 Langer PR, Waldrop AA, Ward DC. Enzymatic synthesis of biotin-labelled polynucleotides: Novel nucleic acid affinity probes. *Proc Natl Acad Sci USA* 1981;78:6633–7.

17 O'Farrell P. Replacement synthesis method of labelling DNA fragments. *BRL Focus* 1981;3:1–3.

18 Maxam AM, Gilbert W. Sequencing end-labelled DNA with base-specific chemical cleavages. In: Grossman L, Muldave K, eds. *Methods in Enzymology*, 65:499. New York, Academic Press, 1980.

19 Leary JJ, Brigati DJ, Ward DC. Rapid and sensitive colorimetric method for visualizing biotin-labelled DNA probes hybridized to DNA or RNA immobilised on nitrocellulose bio-blots. *Proc Natl Acad Sci USA* 1983; 80:4045–9.

20 Chan VTW, Fleming KA, McGee JO. Detection of sub-pigogram quantities of specific DNA sequences on blot hybridization with biotinylated probes. *Nucleic Acids Res* 1985;13:8083–8.

21 Murasugi A, Wallace RB. Biotin-labelled oligonucleotides: Enzymatic synthesis and use as hybridization probes. *DNA* 1984;3:269–77.

22 Chu BCF, Wahl GM, Orgel LE. Derivatization of unprotected oligonucleotides. *Nucleic Acids Res* 1983;11:6513–29.

23 Jablonski E, Moomaw EW, Tullis RH, Ruth JL. Preparation of oligodeoxynucleotide-alkaline phosphatase conjugates and their use as hybridization probes. *Nucleic Acids Res* 1986;14:6115–28.

24 Virtanen M, Palva A, Laaksonen M, Halonen P, Soederlund H, Ranki M. Novel test for rapid viral diagnosis: Detection of adenovirus in nasopharyngeal specimens by means of nucleic acid sandwich hybridization. *Lancet* 1983;i:381–3.

25 Jablonski EG. Detection systems for hybridization reactions. In: Tenover FC, ed. *DNA Probes in Infectious Diseases*, pp15–30. Boca Raton, Florida, CRC Press, 1989.

26 Rotbart HA, Levin MJ. Progress towards the development of a pan-enteroviral nucleic acid probe. In: Tenover FC, ed. *DNA Probes for Infectious Diseases*, pp193–209. Boca Raton, Florida, CRC Press, 1989.

27 Schrier RD, Nelson JA, Oldstone MBA. Detection of human cytomegalovirus in peripheral blood lymphocytes in a natural infection. *Science* 1985;230:1048–51.

28 Ambinder RF, Charache P, Staal S, *et al*. The vector homology problem in diagnostic nucleic acid hybridization of clinical specimens. *J Clin Microbiol* 1986;24:16–20.

29 Saiki RK, Scharf S, Faloona F, *et al*. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985;230:1350–4.

30 Erlich HA, Gelfand DH, Saiki RK. Specific DNA amplification. *Nature* 1988;331:461–2.

31 Abbott MA, Poiesz BJ, Byrne BC, Kwok S, Sninsky JJ, Ehrlich GD. Enzymatic gene amplification: Qualitative and quantitative methods for detecting proviral DNA amplified *in vitro*. *J Infect Dis* 1988;158:1158–69.

32 Fields BN, Knipe DM. Virology, second ed. New York, Raven Press, 1990.

33 Redfield DC, Richman DD, Albanil S, Oxman MN, Wahl GM. Detection of herpes simplex virus in clinical specimens by DNA hybridization. *Diagn Microbiol Infect Dis* 1983;1: 117–28.

34 Richmann DD, Cleveland PH, Redfield DC, Oxman MN, Wahl GM. Rapid viral diagnosis. *J Infect Dis* 1984; 149:298–310.

35 Schuster V, Matz B, Wiegand H, Polack A, Corsten B, Neumann-Haefelin D. Nucleic acid hybridization for detection of herpes viruses in clinical specimens. *J Med Virol* 1986;19:277–86.

36 Rooney JF, Felser JM, Ostrove JM, Straus SE. Acquisition of genital herpes from an asymptomatic sexual partner. *N Engl J Med* 1986;314:1561–4.

37 Croen KD, Ostrove JM, Dragovic LJ, Smialek JE, Straus SE. Latent herpes simplex virus in human trigeminal ganglia: detection of an immediate-early gene "anti-sense" transcript by *in situ* hybridizaton. *N Engl J Med* 1987;317:127–32.

38 Stevens JG, Wagner EK, Devi-Rao GB, Cook ML, Feldman LT. RNA complementary to a herpesvirus alpha gene mRNA is prominent in infected neurons. *Science* 1987;235:1056–9.

39 Peden, K, Mounts P, Hayward GS. Homology between mammalian cell DNA sequences and human herpesvirus genomes detected by a hybridizaton procedure with high-complexity probe. *Cell* 1982;31:81–87.

40 Jones TR, Parks CL, Spector DJ, Hyman RW. Hybridization of herpes simplex virus DNA and human ribosomal DNA and RNA. *Virology* 1985;144:384–97.

41 de Villiers EM. Heterogeneity of the human papillomavirus group (Minireview). *J Virol* 1989;63:4898–903.

42 Crum CP, Ikenberg H, Richart RM, Gissmann L. Human papillomavirus type 16 and early cervical neoplasia. *N Engl J Med* 1984;310:880–3.

43 Crum CP, Mitao M, Levine RU, Silverstein S. Cervical papillomaviruses segregate within morphologically distinct precancerous lesions. *J Virol* 1985;54:675–81.

44 Beckmann AM, Kiviat NB, Daling JR, Sherman KJ, McDougall JK. Human papillomavirus type 16 in multifocal neoplasias of the female genital tract. *Int J Gynecol Pathol* 1988;7:39–47.

45 Ferenczy A, Mitao M, Nagai N, Silverstein SJ, Crum CP. Latent papillomavirus and recurring genital warts. *N Engl J Med* 1985;313:784–8.

46 Wickenden C, Steele A, Malcolm ADB, Coleman DV. Screening for wart virus infection in normal and abnormal cervices by DNA hybridization of cervical scrapes. *Lancet* 1987;i: 65–67.

47 Lorincz AT, Lancaster WD, Kurman RJ, Jenson AB, Temple GF. Characterization of human papillomaviruses in cervical neoplasias and their detection in routine clinical screening. In: Peto R, zur Hausen H, eds; *Viral Etiology of Cervical Cancer*, pp225–36. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1986.

48 Toon PG, Arrand JR, Wilson LP, Sharp DS. Human papillomavirus infection of the uterine cervix of women without cytological signs of neoplasia. *Br Med J* 1986; 293:1261–4.

49 Gergely L, Czegledy D, Hernady Z. Human papillomavirus frequency in normal cervical tissue. *Lancet* 1987;ii:513.

50 DeVilliers EM, Wagner D, Schneider A, *et al*. Human papillomavirus infections in women with and without abnormal cervical cytology. *Lancet* 1987;ii:703–5.

51 Young LS, Bevan IS, Johnson MA, *et al*. The polymerase chain reaction: A new epidemiological tool for investigating cervical human papillomavirus infection. *Br Med J* 1989;298:14–8.

52 Tidy JA, Parry GCN, Ward P, *et al*. High rate of human papillomavirus type 16 infection in cytologically normal cervices. *Lancet* 1989;i:434.

53 Munoz N, Bosch X, Kaldor JM. Does human papillomavirus cause cervical cancer? The state of the epidemiological evidence. *Br J Cancer* 1988;57:1–5.

54 Editorial. Human papillomaviruses and cervical cancer: A fresh look at the evidence. *Lancet* 1987;i:725–6.

55 International Agency for Research on Cancer. Human papillomavirus and cervical cancer. *Lancet* 1988;i:756–7.

56 Editorial. Human papillomaviruses and the polymerase chain reaction. *Lancet* 1989;i:1051–2.

57 Reynolds DW, Stagno S, Stubbs KG, *et al*. Inapparent congenital cytomegalovirus infection with elevated cord IgM levels: Causal relation with auditory and mental deficiency. *N Engl J Med* 1974;290:291–6.

58 Griffith PD, Panjwani DD, Stirk PR, *et al*. Rapid diagnosis of cytomegalovirus infection in immunocompromised patients by detection of early antigen fluorescent foci. *Lancet* 1984;ii:1242–5.

59 Spector SA, Spector DH. The use of DNA probes in studies of human cytomegalovirus. *Clin Chem* 1985;31:1514–20.

60 Myerson D, Hackman RC, Nelson JA, Ward CC, McDougall JK. Widespread presence of histologically occult cytomegalovirus. *Hum Pathol* 1984;15:430–9.

61 Hsia K, Spector DH, Lawrie J, Spector SA. Enzymatic amplification of human cytomegalovirus sequences by polymerase chain reaction. *J Clin Microbiol* 1989;27: 1802–9.

62 Ulrich PP, Busch MP, El-Beik T, *et al*. Assessment of human

immunodeficiency virus expression in cocultures of peripheral blood mononuclear cells from healthy seropositive subjects. *J Med Virol* 1988;25:1–10.

63 Stoeckl E, Barrett N, Heinz FX, *et al.* Efficiency of the polymerase chain reaction for the detection of human immunodeficiency virus type 1 (HIV-1) DNA in the lymphocytes of infected persons: Comparison to antigenenzyme-linked immunosorbent assay and virus isolation. *J Med Virol* 1989;29:249–55.

64 Jackson JB, Kwok SY, Sninsky JJ, *et al.* Human immunodeficiency virus type 1 detected in all seropositive symptomatic and asymptomatic individuals. *J Clin Microbiol* 1990;28:16–9.

65 Harper ME, Marselle LM, Gallo RC, Wong-Staal F. Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes and peripheral blood from infected individuals by *in situ* hybridization. *Proc Natl Acad Sci USA* 1986;83:772–6.

66 Busch MP, Rajagopalan MS, Gantz DM, Fu S, Steimer KS, Vyas GN. *In situ* hybridization and immunochemistry for improved assessment of HIV cultures. *Am J Clin Pathol* 1987;88:673–80.

67 Stoler MH, Eskin TA, Benn S, Angerer RC, Angerer LM. Human T-cell lymphotropic virus type III infection of the central nervous system: A preliminary *in situ* analysis. *JAMA* 1986;256:2360–4.

68 Kwok SK, Mack DH, Mullis KB, *et al.* Identification of human immunodeficiency virus sequences by using *in vitro* enzymatic amplification and oligomer cleavage detection. *J Virol* 1987;61:1690–4.

69 Ou CY, Kwok S, Mitchell SW, *et al.* DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. *Science* 1988;239:295–9.

70 Ranki A, Valle SL, Krohn M, *et al.* Long latency precedes overt seroconversion in sexually transmitted human immunodeficiecy virus infection. *Lancet* 1987;ii:589–93.

71 Ward JW, Holmberg SD, Allen JR, *et al.* Transmission of human immunodeficiency virus (HIV) by blood transfusions screened as negative for HIV antibody. *N Engl J Med* 1988;318:473–8.

72 Loche M, Mach B. Identification of HIV-infected seronegative individuals by a direct diagnostic test based on hybridization to amplified viral DNA. *Lancet* 1989;ii:418–21.

73 Pezzella M, Rossi P, Lombardi V, *et al.* HIV viral sequences in seronegative people at risk detected by *in situ* hybridization and polymerase chain reaction. *Br Med J* 1989;298:713–6.

74 Pezzella M, Caprilli F, Vonesch N, *et al.* Detection of HIV genome in HIV antibody negative men. *Genitourin Med* 1989;65:293–9.

75 Rogers MF, Ou CY, Rayfield M, *et al.* Use of the polymerase chain reaction for early detection of the proviral sequences of human immunodeficiency virus in infants born to seropositive mothers. *N Engl J Med* 1989;320:1649–54.

76 Paterlini P, Lallemant-LeCoeur S, Lallemant M, *et al.* Polymerase chain reaction for studies of mother to child transmission of HIV1 in Africa. *J Med Virol* 1990;30:53–7.

77 Horsburgh CR, Ou CY, Jason J, *et al.* Duration of human immunodeficiency virus infection before detection of antibody. *Lancet* 1989;ii:637–40.

78 Spector SA, Hsia K, Denaro F, Spector CH. Use of molecular probes to detect human cytomegalovirus and human immunodeficiency virus. *Clin Chem* 1989;35:1581–7.

79 Meyerhans A, Cheynier R, Albert J, *et al.* Temporal fluctuations in HIV quasispecies *in vivo* are not reflected by sequential HIV isolations. *Cell* 1989;58:901–10.

80 Kuo G, Choo QL, Alter HJ, *et al.* An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 1989;244:362–4.

81 Ono Y, Onda H, Sasada R, Igarashi K, Sugino Y Wishioka K. The complete nucleotide sequences of cloned hepatitis B virus DNA subtype adv and adw. *Nucleic Acids Res* 1983;11:1747–57.

82 Scotto J, Hadchouel M, Hery C, Yvart J, Tiollais P, Brechot C. Detection of hepatitis B virus DNA in serum by a simple spot hybridization technique: Comparison with results for other viral markers. *Hepatology* 1983;3:279–84.

83 Blum HE, Stowring, L, Figus, A, Montgomery CK, Haase AT, Vyas GN. Detection of hepatitis B virus DNA in hepatocytes, bile duct epithelium and vascular elements by *in situ* hybridization. *Proc Natl Acad Sci USA* 1983;80:6685–8.

84 Dejean A, Lugassy C, Zafrani S, Tiollais P, Brechot C.

Detection of hepatitis B virus DNA in pancrease, kidney and skin of two human carriers of the virus. *J Gen Virol* 1984;65:651–5.

85 Elfassi E, Romet-Lemonne JL, Essex M, McLane MF, Haseltine WA. Evidence of extrachromosomal forms of hepatitis B virus DNA in a bone marrow culture obtained from a patient recently infected with hepatitis B virus. *Proc Natl Acad Sci USA* 1984;81:3526–530.

86 Pontisso P, Poon MC, Tiollais P, Brechot C. Detection of hepatitis virus DNA in mononuclear blood cells. *Br Med J* 1984;288:1563–6.

87 Laure F, Zagury D, Saimont AG, Gallo RC, Hahn BH, Brechot C. Hepatitis B virus DNA sequences in lymphoid cells from patients with AIDS and AIDS-related complex. *Science* 1985;229:561–3.

88 Morichika S, Hada H, Arima T, Togawa K, Watanabe M, Nagashima H. Hepatitis B virus DNA replication in peripheral blood mononuclear cells. *Lancet* 1985;ii:1431.

89 Hoar DI, Bowen T, Matheson D, Poon MC. Hepatitis B virus DNA is enriched in polymorphonuclear leucocytes. *Blood* 1985;66:1251–3.

90 Noonan CA, Yoffe B, Mansell PWA, Melnick JL, Hollinger FB. Extrachromosomal sequences of hepatitis B virus DNA in peripheral blood mononuclear cells of AIDS patients. *Proc Natl Acad Sci USA* 1986;83:5698–702.

91 Yoffe B, Noonan CA, Melnick JL, Hollinger FB. Hepatitis B virus DNA in mononuclear cells and analysis of cell subsets for the presence of replicative intermediates of viral DNA. *J Infect Dis* 1986;153:471–7.

92 Lin HJ, Wu PC, Lai CL. An oligonucleotide probe for the detection of hepatitis B virus DNA in serum. *J Virol Methods* 1987;15:139–49.

93 Lin HJ, Chung HT, Lai CL, Leong S, Tam OS. Detection of supercoiled hepatitis virus DNA and related forms by means of molecular hybridization to an oligonucleotide probe. *J Med Virol* 1989;29:284–8.

94 Suzuki H, Ichida F, Fujisawa K, *et al.* Double blind controlled study of the antiviral effect of human interferon B on chronic active hepatitis. In: Zuckerman AJ, ed. *Viral Hepatitis and Liver Diseases* pp 864–867. New York, Alan R Liss, 1988.

95 Smedile A, Bergmann KF, Baroudy BM, *et al.* Riboprobe assay for HDV RNA: A sensitive method for the detection of the HDV genome in clinical serum samples. *J Med Virol* 1990;30:20–4.

96 Weiner AJ, Kuo G, Bradley DW, *et al.* Detection of hepatitis C viral sequences in non-A, non-B hepatitis. *Lancet* 1990;i:1–3.

97 Anderson MJ, Jones SE, Fisher-Hoch SP, *et al.* The human parvovirus, the cause of erythema infectosum (Fifth disease)? *Lancet* 1983;i:1378.

98 White DG, Woolf AD, Mortimer PP, Cohen BJ, Allen GE, Connolly JH. Human parvovirus arthropathy. *Lancet* 1985;i:419–21.

99 Pattison JR, Jones SE Hodgson.J, *et al.* Parvovirus infections and hypoplastic crises in sickle cell anaemia. *Lancet* 1981;i:664–5.

100 Rao KRP, Patel AR, Anderson MJ, Hodgson J, Jones SE, Pattison JR. Infection with parvovirus-like virus and aplastic crisis in chronic haemolytic anaemia. *Ann Int Med* 1983;98:930–2.

101 Brown T, Anand A, Richie LD, Clewley JP, Reid TMS. Intrauterine parvovirus infection associated with hydrops fetalis. *Lancet* 1984;ii:1033–4.

102 Knott PD, Welply GAC, Anderson MJ. Serologically proved intrauterine infection with parvovirus. *Br Med J* 1984;289:1660.

103 Gray ES, Anand A, Brown T. Parvovirus infections in pregnancy. *Lancet* 1986;i:208.

104 Bond PR, Caul EO, Usher J, Cohen BJ, Clewley JP, Field AM. Intrauterine infection with human parvovirus. *Lancet* 1986;i:449–50.

105 Anand A, Gray ES, Brown T, Clewley JP, Cohen BJ. Human parvovirus infection in pregnancy and hydrops fetalis. *N Engl J Med* 1987;316:183–6.

106 Anderson MJ, Jones SE, Minson AC. Diagnosis of human parvovirus infection by dot-blot hyridization using cloned viral DNA. *J Med Virol* 1985;15:163–72.

107 Clewley JP. Detection of human parvovirus using a molecularly cloned probe. *J Med Virol* 1985;15:173–81.

108 Cohen BJ. Laboratory tests for the diagnosis of infection with B19 virus. In: Pattison JR, ed *Parvoviruses and Human Disease* 1986. 69–83. Boca Raton, Florida, CRC Press.

109 Clewley JP, Cohen BJ, Field AM. Detection of parvovirus B19 DNA, antigen and particles in the human fetus. *J Med Virol*

1987;23:367–76.

110 Clewley JP. Polymerase chain reaction assay of parvovirus B19 DNA in clinical specimens. *J Clin Microbiol* 1989; 27:2647–51.

111 Koch WC, Adler SP. Detection of human parvovirus B19 DNA by using the polymerase chain reaction. *J Clin Microbiol* 1990;28:65–69.

112 Holmes KK. The chlamydia epidemic. *JAMA* 1981; 245:1718–23.

113 Schachter J. Epidemiology of *Chlamydia trachomatis* infections. In: de la Maza LM, Peterson EM, eds *Medical Virology*, 1983. 111–120. New York, Elsevier Science Publ.

114 Bell TA, Grayston JT. Centers for Disease Control guidelines for prevention and control of *Chlamydia trachomatis* infection. *Ann Intern Med* 1986;104:524–6.

115 Palva A, Jousimies-Somer H, Saikku P, Vaeaenaenen P, Soederlund H, Ranki M. Detection of *Chlamydia trachomatis* by nucleic acid sandwich hybridization. *FEMS Microbiol Lett* 1984;23:83–89.

116 Hyypiae T, Larsen SH, Stahlberg T, Terho P. Analysis and detection of chlamydial DNA. *J Gen Microbiol* 1984;130:3159–64.

117 Hyypiae T, Jalava A, Larsen SH, Terho P, Hukkanen V. Detection of *Chlamydia trachomatis* in clinical specimens by nucleic acid spot hybridization. *J Gen Microbiol* 1985;131:975–8.

118 Horn JE, Hammer ML, Falkow S, Quinn TC. Detection of *Chlamydia trachomatis* in tissue culture and cervical scraping by *in situ* hybridization. *J Infect Dis* 1986;153:1155–9.

119 Palva A, Korpela K, Lassus A, Ranki M. Detection of *Chlamydia trachomatis* from genitourinary specimens by improved nucleic acid sandwich hybridization. *FEMS Microbiol Lett* 1987;40:211–8.

120 Dean D, Palmer L, Pant CR, Courtright P, Falkow S, O'Hanley P. Use of a *Chlamydia trachomatis* DNA probe for detection of ocular Chlamydiae. *J Clin Microbiol* 1989;27:1062–7.

121 LeBar W, Herschman B, Jemal C, Pierzchala J. Comparison of DNA probe, monoclonal antibody enzyme immunoassay, and cell culture for the detection of *Chlamydia trachomatis*. *J Clin Microbiol* 1989;27:826–8.

122 Peterson EM, Oda R, Alexander R, Greenwood DR, de la Maza LM. Molecular techniques for the detection of *Chlamydia trachomatis*. *J Clin Microbiol* 1989;27:2359–63.

123 Perine PL, Totten PA, Holmes KK, *et al.* Evaluation of DNA hybridization method for detection of African and Asian strains of *Neisseria gonorrhoea* in men with urethritis. *J Infect Dis* 1985;152:59–63.

124 Schachter J. Rapid diagnosis of sexually transmitted diseases—speed has a price. *Diagn Microbiol Infect Dis* 1986; 4:185–9.

125 Takiff HE, Seidlin M, Krause P, Rooney J, Brandt C, Rodriguez W, Yolken R, Straus SE. Detection of enteric adenoviruses by dot-blot hybridization using a molecularly cloned viral DNA probe. *J Med Virol* 1985; 16:107–18.

126 Kidd AH, Harley EH, Erasmus MJ. Specific detection and typing of adenovirus type 40 and 41 in stool specimens by dot blot hybridization. *J Clin Microbiol* 1985;22:934–9.

127 Hyypiae T. Detection of adenovirus in nasopharyngeal specimens by radoactive and non-radioactive DNA probes. *J Clin Microbiol* 1985;21:730–3.

128 Lehtomaki K, Julkunen I, Sandelin K, *et al.* Rapid diagnosis of respiratory adenovirus infections in young adult men. *J Clin Microbiol* 1986;24:108–11.

129 Hyman RW, Ecker JK, Tenser RB. Varicella-zoster RNA in human trigeminal ganglia. *Lancet* 1983;ii:814–6.

130 Seidlin M, Takiff HE, Smith HA, Hay J, Straus SE. Detection of varicella-zoster virus by dot-blot hybridization using a molecularly cloned viral DNA probe. *J Med Virol* 1984;13:53–61.

131 Sixbey JW, Shirley PS. Nucleic acid probes for the detection of Epstein Barr virus. In: Tenover FC, ed *DNA Probes for Infectious Diseases*, 1989, 127–34. Boca Raton, Florida, CRC Press.

132 Estes MK, Tanaka T. Nucleic acid probes for rotavirus detection and characterization. In: DNA Probes for Infectious Diseases, Tenover FC, ed 1989, 79–100. Boca Raton, Florida, CRC Press.

133 Tenover FC. DNA probes for bacterial stool pathogens. In: Tenover FC, ed *DNA Probes for Infectious Diseases,* 1989, 53–63. Boca Raton, Florida, CRC Press.

134 Kaper JB, Morris Jr JG, Nishibuchi M. DNA probes for pathogenic *vibrio* species. In: *DNA Probes for Infectious Diseases,* Tenover FC, ed 1989, 65–77. Boca Raton, Florida, CRC Press.

135 Shaw SB. DNA probes for the detection of *legionella* species, *Mycoplasma pneumoniae* and members of the *Mycobacterium tuberculosis* complex. In: Tenover FC, ed *DNA Probes for Infectious Diseases*, 1989, 101–17. Boca Raton, Florida, CRC Press.

136 Ihler GM, Rice-Fichl AC. Detection of hemotropic parasites by DNA hybridization. In: Tenover FC, ed *DNA Probes for Infectious Diseases*, 1989, 233–47. Boca Raton, Florida, CRC Press.

137 Wirth DF, Rogers WO, Dourado H, Albuquerque B. Leishmaniasis: DNA probes for diagnosis. In: Tenover FC, ed *DNA Probes for Infectious Diseases*, 1989, 249–58. Boca Raton, Florida, CRC Press.

138 Tenover FC. DNA probes and antimicrobial susceptibility testing. In: Tenover FC, ed *DNA Probes for Infectious Diseases*, 1989, 259–68. Boca Raton, Florida, CRC Press.